potential role for both protein-protein and protein-RNA interactions in targeting LC3 to the nucleus and nucleolus.

Voltage-gated Na Channels

2893-Pos Board B323
Resting State of S4 Identified for Each Domain of Nav1.2 using Omega Current Technique
Claudia Lehmann, Hansjakob Heldstab, Nikolaus G. Greeff.
Biophysics Institute Greeff, Uetikon am See, Switzerland.
During gating transitions the voltage sensor S4 slides through the narrow so-called gating pore. The positively charged amino acids of S4, arginine (R) or lysine (K) sense the transmembranal electric field and promote S4 in or out. If one or more long R or K is replaced by the short neutral glutamine (Q), a leak (omega-) pore is created through which a leak current, the omega current will flow when S4 is at the appropriate position. The occupancy of this leaky position can then be electrically monitored. Rat brain sodium channels Nav1.2 were studied at high expression in X. laevis oocytes with two-electrode voltage clamping at strong hyperpolarization to force S4 into the resting state. Mutant channels with single gaps R1Q, R2Q or R3Q as well as with double gaps RRn,n+1QQ were tested for the presence of omega leaks. We found unambiguous omega currents for double gaps in domain DI (RR12QQ), DII (RR12QQ), DIII (RR23QQ) and DIV (RR12QQ), indicating the resting position of S4 in each domain. These findings are in contrast to skeletal muscle sodium channels Nav1.4 where single gap omega leaks are reported for DII and DIII. However, our single gap mutants in Nav1.2 produced only very small leak currents similar to artifacts sometimes also occurring at wild-type channels at very strong hyperpolarizing pulses and at least ten times smaller than those with double gaps. Based on this study, we currently use our double gap channel constructs as a tool to selectively investigate which S4 of the four domains I-IV are immobilized by inactivation.

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Selective Immobilization of S4 in Domain III and IV of Rat Brain Nav1.2 Shown by Omega Currents
Nikolaus Greeff, Hansjakob Heldstab, Claudia Lehmann.
Biophysics Institute Greeff, Uetikon am See, Switzerland.
Recently, we have identified the minimally necessary number of mutations of the outer positively charged arginine (R) or lysine (K) to glutamine (Q) in the voltage sensor S4 of each domain, producing an inward omega leak current in the resting state. In all four domains a double gap (RRn,n+1QQ) gave distinct omega currents. In this study we use these double gap channel constructs as a tool to investigate which S4 of the four domains I-IV is immobilized by inactivation. The recovery time constant of sodium current after inactivation was measured with a classical double pulse protocol for a wide range of recovery potentials from −100 to −240 mV. In addition, the onset of the omega current at the same recovery potentials was measured twofold: without and with an inactivating prepulse. We found that the onset of omega current was fast and not affected by inactivation in domain I and II; however, in domain III and IV the onset was fast without prepulse but was slowed after the inactivating prepulse. The return to the resting state seems to be hindered due to immobilization. The time constant of the recovery of omega current matches well the recovery of sodium current over the wide potential range studied. We corroborated our results by using the mutation R4H in S4DIV, which slows the sodium current recovery about twentyfold (Kühn and Greeff, 1999). Adding the mutation R4H in S4DIV to our double gap constructs, we found that the omega current was also slowed by the same factor. This suggests that the same mechanism which keeps the alpha pore closed for ionic current in inactivated channels would also hinder the return of S4III and S4IV to the resting position.

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Voltage Sensor Domains and Closed-State Inactivation in Sodium Channels
James R. Groome.
Biological Sciences, Idaho State University, Pocatello, ID, USA.
Sodium channels enter into a state of fast inactivation after opening, or directly from closed states. We examined the roles of the four voltage sensor domains in hNaV1.4 in closed-state fast inactivation using a mutagenesis approach. Charge reversing mutations of outer arginine residues in domains I, III and IV depolarized the steady-state fast inactivation curve and accelerated entry. Similar effects on closed-state fast inactivation were observed for charge-reversing mutations of inner negative charges in domains I and IV, suggesting that electrostatic interaction of these residues limits S4 translocation in response to sub-threshold depolarization. This work was supported by NIH 2P20GM103408 to ISU.

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Gating Pore Currents are Common Defects of Two Nav1.5 Mutations in Patients with Mixed Arrhythmias and Dilated Cardiomyopathy
Adrien Moreau[1], Pascal Gosselin-Badaroudine[1], Lucie Delemotte[2], Michael L. Klein[2], Mohamed Chahine[1,3].
[1]Electrophysiology, CRIUSMQ, Quebec, QC, Canada, [2]Institute of Computational Molecular Science, Philadelphia, PA, USA, [3]Department of Medicine, Université Laval, Quebec, QC, Canada.
The gating pore current, also called omega current, consists of a cation leak through the typically non-conductive voltage sensor domain (VSD) of voltage gated ion channels (VGIC). While the study of gating pore current refined the knowledge of the structure and the function of VGIC, their implication in cardiac disorders has not been established. Two Nav1.5 mutations (R222Q and R225W) localized in the VSD are associated with complex arrhythmias and dilated cardiomyopathy. Using the patch clamp technique, in-silico mutagenesis and molecular dynamic simulations, we tested the hypothesis that these two mutations may generate gating pore currents potentially accounting for their atypical clinical phenotypes. Our findings suggest that the gating pore current generated by the R222Q and R225W mutations could constitute the yet unrevealed pathological mechanism linking Nav1.5 VSD mutations with cardiac arrhythmias and dilatation of cardiac chambers in humans.

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Interaction of the Cardiac Sodium Channel Alpha-Subunits Leads to Coupled Gating Properties
Jérôme Clatot, Haiyan Liu, Eckard Ficker, Isabelle Deschênes.
Case Western Reserve University, Case Western Reserve University, OH, USA.
Objective: The cardiac sodium channel has been linked to cardiac arrhythmias. We have shown the existence of dominant-negative mutations in Brugada Syndrome due to interactions between alpha-subunits. Here we investigated the stoichiometry of the interaction and whether the interaction leads to coupled gating properties.
Methods: Single-molecule pull-down experiments and blue native gels have been performed to study the stoichiometry of the interaction, while FRET/TIRF experiments were performed to investigate the interaction at the cell surface. Biophysical properties were studied by patch-clamp analysis in the whole-cell configuration.
Results: Biochemistry results support the dimerization of alpha-subunits. FRET/TIRF experiments showed interacting channels at the plasma membrane. Also, we investigated if the dimerization of the channel leads to biophysical consequences. To do so, we used different mutants leading to specific biophysical. R1860X and R1629Q mutants were used for inactivation and R878C for gating deficiency. When cells expressed WT and R1629Q mutant channels, inactivation properties behaved more closely to the WT contrarily to what would be expected of 2 channels working independently. In addition, when R1629Q was coepxressed with the gating deficient mutant R878C we showed a significant improvement of the R1629Q inactivation properties, even though R878C is not conducting. We then used a truncated channel R1860X and once again the coexpression with R878C significantly improved the inactivation defect, which was not the case with the expression of a C-terminus fragment alone. This, strongly suggest that the presence of the full length channel could rescue the inactivation defect of the delta-Cter channel.
Conclusions: Our data indicate that the alpha-subunits of the cardiac sodium channel present coupled gating properties due to the formation of dimers.

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Superresolution Microscopy Reveals Sodium Channel Localization within Intercalated Disk Microdomains: Implications for Ephaptic Coupling
Rengasayee Veeraraghavan[1], Joyce Lin[2], James P. Keener[3], Steven Poelzing[1], Robert G. Gourdie[1].
[1]Virginia Tech Carilion Research Institute, Virginia Polytechnic University, Roanoke, VA, USA, [2]California Polytechnic State University, San Luis Obispo, CA, USA, [3]Dept. of Mathematics, University of Utah, Salt Lake City, UT, USA.
Pore-forming (Nav1.5) and auxiliary (β1; SCN1b) subunits of cardiac sodium channels are enriched at the cardiomyocyte intercalated disk (ID). Mathematical models suggest that this may facilitate conduction via ephaptic mechanisms. We previously demonstrated anisotropic conduction slowing during acute interstitial edema (AIE), possibly due to weakened ephaptic coupling. Here we assessed Nav1.5 and β1 localization to ID microdomains using